

Microarray analysis of whole genome expression of intracellular Mycobacterium tuberculosis

Article (Accepted Version)

Waddell, S J and Butcher, P D (2007) Microarray analysis of whole genome expression of intracellular Mycobacterium tuberculosis. *Current Molecular Medicine*, 7 (3). pp. 287-296. ISSN 1566-5240

This version is available from Sussex Research Online: <http://sro.sussex.ac.uk/id/eprint/7632/>

This document is made available in accordance with publisher policies and may differ from the published version or from the version of record. If you wish to cite this item you are advised to consult the publisher's version. Please see the URL above for details on accessing the published version.

Copyright and reuse:

Sussex Research Online is a digital repository of the research output of the University.

Copyright and all moral rights to the version of the paper presented here belong to the individual author(s) and/or other copyright owners. To the extent reasonable and practicable, the material made available in SRO has been checked for eligibility before being made available.

Copies of full text items generally can be reproduced, displayed or performed and given to third parties in any format or medium for personal research or study, educational, or not-for-profit purposes without prior permission or charge, provided that the authors, title and full bibliographic details are credited, a hyperlink and/or URL is given for the original metadata page and the content is not changed in any way.

Published in final edited form as:

Curr Mol Med. 2007 May ; 7(3): 287–296.

Microarray Analysis of Whole Genome Expression of Intracellular *Mycobacterium tuberculosis*

Simon J. Waddell* and Philip D. Butcher

Medical Microbiology, Division of Cellular & Molecular Medicine, St. George's University of London, Cranmer Terrace, Tooting, London, SW17 0RE, UK

Abstract

Analysis of the changing mRNA expression profile of *Mycobacterium tuberculosis* though the course of infection promises to advance our understanding of how mycobacteria are able to survive the host immune response. The difficulties of sample extraction from distinct mycobacterial populations, and of measuring mRNA expression profiles of multiple genes has limited the impact of gene expression studies on our interpretation of this dynamic infection process. The development of whole genome microarray technology together with advances in sample collection have allowed the expression pattern of the whole *M. tuberculosis* genome to be compared across a number of different *in vitro* conditions, murine and human tissue culture models and *in vivo* infection samples. This review attempts to produce a summative model of the *M. tuberculosis* response to infection derived from or reflected in these gene expression datasets. The mycobacterial response to the intracellular environment is characterised by the utilisation of lipids as a carbon source and the switch from aerobic/microaerophilic to anaerobic respiratory pathways. Other genes induced in the macrophage phagosome include those likely to be involved in the maintenance of the cell wall and genes related to DNA damage, heat shock, iron sequestration and nutrient limitation. The comparison of transcriptional data from *in vitro* models of infection with complex *in vivo* samples, together with the use of bacterial RNA amplification strategies to sample defined populations of bacilli, should allow us to make conclusions about *M. tuberculosis* physiology and host microenvironments during natural infection.

Keywords

Intracellular; expression; microarray; transcriptomics; mycobacterium; macrophage; host pathogen interactions

INTRODUCTION

The mycobacterial transcriptome consists of the mRNA content of bacilli in a particular environment at a specific time. This represents, subject to additional levels of mRNA and protein regulation, the products required by mycobacteria for continued survival. Little is known of the events that occur at the gene expression level in host tissues after infection with *Mycobacterium tuberculosis*. However the development of techniques such as subtractive hybridisation, quantitative RT-PCR and microarray technologies have allowed the transcriptional responses of both host and pathogen to be explored during infection. Thus

it is possible to determine the immediate genome-wide response (as measured by mRNA expression) of mycobacteria to a stimulus or environment. The comparison of mycobacterial mRNA expression patterns from intracellular infection models provides a snapshot of the network of interactions between bacilli and the external environment. These transcriptional datasets can be used to (a) help understand the changing metabolism of infecting bacilli, which may in turn provide insights into the host microenvironments encountered by bacilli; (b) identify pathogenic mechanisms used by *M. tuberculosis* to evade and modulate the immune response; and (c) define the interactions between host and pathogen throughout the multi-factorial and dynamic process of infection. This review outlines the mycobacterial response to infection derived from the mRNA expression patterns of *M. tuberculosis* extracted from intracellular environments.

***M. tuberculosis* Pathogenesis**

The complex pathogenesis of *M. tuberculosis* is influenced by a multitude of factors including host and bacterial genetic backgrounds, immunological status and environmental circumstances. The earliest encounter between infecting bacilli and the immune system is likely to be with alveolar macrophages; that some bacilli survive this initial contact provides a relevant starting point to begin investigating host-*M. tuberculosis* interactions.

Primary infection with *M. tuberculosis* takes place in the lungs, and may eventually result in pulmonary tuberculosis; however only 5-10% of those individuals infected actually develop clinical disease [1]. On infection a proportion of infecting bacilli are able to survive and successfully multiply within the intracellular environment of the alveolar macrophage. A fundamental step in mycobacterial survival is the halt of phagosome maturation in macrophages after phagocytosis of *M. tuberculosis* [2]. The bacilli therefore avoid destruction in the fused phagolysosome by acidic hydrolases [3], with the pH of mycobacterial-containing phagosomes remaining at 6.5 [4]. Characterisation of the surface markers of *M. tuberculosis*-containing phagosomes reveal that many markers of early endosomes and from the plasma membrane are present, for example mycobacterial phagosomes retain the Rab5 molecule but do not acquire the late endosomal marker Rab7, which is believed to be involved in the transition from early to late endosome [5]. Successful control of *M. tuberculosis* infection is achieved in the lung by the formation of granulomas, where activated macrophages surround the site of infection preventing further dissemination of bacilli and limiting tissue damage [6]. The human granuloma consists of many different cell types including macrophages, giant cells, fibroblasts, T cells and B cells [7], and requires the orchestration of a range of chemokines, cytokines, adhesins and integrins to coordinate the recruitment, migration and retention of cells to the granuloma [8]. A balance is reached where the mycobacterial infection has been controlled, but the bacilli inside the granuloma have not been destroyed [9]. This situation continues until the immunological balance is shifted in such a way that allows the bacilli to begin successfully multiplying again resulting in pulmonary disease [10].

The immuno-modulatory capacity of *M. tuberculosis* at the interface of bacilli and macrophage has been established for example by the secretion of eukaryotic-like protein kinase G by mycobacteria within the macrophage phagosome [11], and the demonstration that mycobacterial lipids and proteins are trafficked out of infected phagosomes and into uninfected bystander macrophages [12]. The dynamic interaction between mycobacteria and host immune system is also exemplified by the variable contributions of immune cell surface receptors (*e.g.* TLR receptors) to the successful control of *M. tuberculosis* at different stages of infection [13]. Further characterisation of the cross-talk between host immune system and *M. tuberculosis* may reveal novel virulence mechanisms and suggest future innovative treatment strategies.

The metabolic state of bacilli through the course of human infection is largely unknown, especially that of persistent bacilli. However, transcriptional activity has been detected in persistent mycobacteria from *in vivo* murine models of infection using RT-PCR [14, 15]. The inference that persistent bacilli are metabolically active in some way is especially important in the design of new drugs against *M. tuberculosis* [16]. The significance of mycobacterial persistence and drug tolerance has been reviewed by Gomez and McKinney [17]. What is clear however is that the metabolic state of infecting bacilli is likely dependent on a number of factors including the oxygen tension and tissue structure of the surrounding environment in the human lung [18, 19].

A greater understanding of how mycobacteria are able to survive the complex environments encountered during infection will aid novel treatment strategies targeted more specifically at intracellular or persistent bacilli. Expression analyses have the power to inform us how the infecting bacilli are responding to the environments in which they reside, telling us a little about the different niches that mycobacteria occupy and how they are able to adapt. The interactions between *M. tuberculosis* and macrophage have been reviewed recently by Schnappinger *et al.* [20]; McKinney and Gomez [21]; and the immunology of *M. tuberculosis* control is described in detail by Flynn and Chan [7]; and Kaufmann [22]. This review focuses on the mycobacterial transcriptional response to the intracellular environment during early infection, and how the expression profile of *M. tuberculosis* might change with the progression of disease.

The *M. tuberculosis* Transcriptional Response to the Intracellular Environment

The understanding of mycobacterial interactions with the host has been enhanced by expression studies using (a) *in vitro* models, such as how bacilli respond to microaerophilic conditions [23, 24], as reviewed by Bacon and Marsh in this issue; (b) tissue culture models of *M. tuberculosis* infection using murine or human derived macrophages [25, 26]; (c) animal models such as the murine model of *M. tuberculosis* disease [27]; and (d) from human patient samples [19]. Here we review the limited expression data available from intracellular models of infection, concentrating on the studies using whole genome expression analyses but also incorporating data from quantitative RT-PCR, *in situ* hybridisation, promoter trap and subtractive/enrichment hybridisation methodologies. Table (1) details the publications to date that have looked at the *in vivo* expression of multiple *M. tuberculosis* genes, as opposed to mutant or essentiality studies. Mycobacterial gene expression patterns identified in tissue culture or *in vitro* models of infection enable us to dissect out the complex interactions between pathogen and host. We review *M. tuberculosis* expression profiles derived from macrophage infection models in comparison with *in vivo* transcriptional data. Gene expression data may be classified and interrogated by functional category, chromosome position or overlaid onto metabolic pathways. This third approach is the most informative, helping to define the physiological state of infecting mycobacteria, and has been adopted here wherever possible. The major metabolic themes of intracellular *M. tuberculosis* gene expression are detailed below and depicted in Fig. (1).

β -Oxidation of Fatty Acids

The induction of multiple genes in four of the five reactions required for the β -oxidation of fatty acids (*fadD3*, 9, 10, 19, *fadDE5*, 14, 22, 23, 24, 27, 28, 29, 31, *echA19*, *fadB2*, 3, *fadA5*, 6), and the subsequent metabolism of degradation products via the citric and glyoxylate cycles (*icl*, *gltA1*, *Rv1130*) suggests that *M. tuberculosis* utilises a diverse range of fatty acids as a carbon source during intraphagosomal growth [25, 28]. The induction of these genes involved in fatty acid metabolism on infection of macrophages indicates that the phagosomal compartment that bacilli occupy in at least one stage of infection is low in other sources of carbon such as glucose and glycerol [29]. In addition the up-regulation of *pckA*,

encoding a phosphoenolpyruvate carboxykinase, reveals that fatty acids may be converted through gluconeogenesis into sugars. Genes in these fatty acid metabolism pathways (namely *icl* and *pckA*) have also been shown by RT-PCR [30] and microarray analysis [27] to be induced in the mouse lung after infection, and within artificial murine granulomas [31]. The up-regulation of *icl* has also been described on infection of human-derived macrophage-like cells, using SCOTS [32] and subtractive hybridisation [33], and inside non-necrotic human lung granulomas by *in situ* hybridisation [18]. The source of these fatty acids is unknown; it may be that the smorgasbord of complex lipids generated by *M. tuberculosis* is utilised, or host lipids, or a combination of the two depending on the particular microenvironment in which the bacilli find themselves. This pattern of fatty acid utilisation does however appear to be common to all infection models tested. It may therefore represent a fundamental adaptation to intracellular growth and an important target pathway for *M. tuberculosis* drug development.

Respiratory State

Transcriptional changes have been identified in the aerobic and anaerobic respiratory pathways of *M. tuberculosis* during infection. Genes related to aerobic respiration such as the type I NADH dehydrogenase (*nuoABDFIL*), and cytochrome C reductase (*qcrC*) were identified to be repressed inside the macrophage phagosome; in contrast genes encoding products involved in alternative electron acceptor pathways (*frdA* a fumarate reductase, *narX* a nitrate reductase and *ndh* a type II NADH dehydrogenase) were induced [25]. A similar profile of gene expression has been described in the mouse lung during infection by molecular beacon RT-PCR and has lead Shi *et al.* [34] to propose that *M. tuberculosis* may exist in three different respiratory states depending on the microenvironment encountered. During acute infection the energy-efficient type I NADH dehydrogenase (exemplified by the expression of *nuoB*) together with the aa3-cytochrome C complex (assayed by following the expression of *qcrC* and *ctaD*) as a terminal oxidase is utilised. Immune cell activation leading to the generation of nitric oxide blocks this aerobic respiratory pathway and drives *M. tuberculosis* into a transitional respiratory state characterised by the repression of the aa3 cytochrome C terminal oxidase complex (*qcrC*, *ctaD*) and induction of the bd-type terminal oxidase (exemplified by the expression of *cydA*) and nitrate respiration (assayed by following the expression of *narK2*). During chronic murine infection the bd-type terminal oxidase is down-regulated and nitrate is used as the final electron acceptor [34]. This pattern is also reflected in the expression of the *dosR* regulon, a set of around 50 genes induced by hypoxia [23, 35] and nitric oxide treatment [36]. This regulon was induced in *M. tuberculosis* after stimulation of murine macrophages with IFN γ and was dependent on the presence of a functioning murine NOS2 enzyme [25]. The up-regulation of *dosR* and around 20 other members of the regulon was also demonstrated within artificial murine granulomas [31]. Furthermore induction of the *dosR* regulon (assayed using the expression of *hspX*, *Rv2623* and *Rv2626c*) was described to occur in the mouse lung as bacterial numbers began to fall following the induction of the adaptive immune response [37]; a similar profile was also observed for the expression of *Rv1738*, *fdxA*, *acg*, *hspX* and *Rv2626c* in the murine lung [36]. Therefore the *dosR* regulon, mediated by nitric oxide (and/or hypoxia), is induced in the murine model of infection on activation of the immune response and may be part of a mycobacterial response that leads to persistence in the host. The respiratory and metabolic status of persistent bacilli has recently been comprehensively reviewed by Boshoff and Barry [38].

The respiratory state of bacilli during human disease is less well documented. The expression of *narX* coding for a probable nitrate reductase was demonstrated to be expressed in *M. tuberculosis* located in human non-necrotic granulomas [18, 19]; the nitrate reductases *narX*, *narG* and the fumarate reductase, *frdA* were also found to be induced in the human

pericavity and distant lung of tuberculosis patients [19]. Additionally the induction of both aerobic and anaerobic genes in human lung tissue [19] suggests that the respiratory status of infecting bacilli is dependent on the microenvironment encountered. The induction of the *dosR* regulon has not been fully demonstrated in human infection, although *hspX* has been reported to be up-regulated in the human lung [30]. The role of nitric oxide in the control of *M. tuberculosis* infection in man is unclear, however the ability of *M. tuberculosis* to utilise several respiration pathways and the induction of alternative electron acceptors during infection provide possible drug development targets. Transcriptomic analysis of smaller more distinct populations of bacilli during natural infection may help to elucidate the variable respiratory state of *M. tuberculosis* in the human lung.

Inextricably linked to respiration state are the energy requirements of the infecting bacilli; genes encoding ATP synthase subunits (*atpABD-H*) were down-regulated in the murine macrophage phagosome and this pattern was repeated in naïve, IFN γ -activated or NOS2-deficient macrophages compared to *in vitro* growth [25]. Furthermore *atpA* and *atpD* were repressed during chronic infection of immuno-competent mice [34]. This repression of ATP-synthases *in vivo* is likely to simply reflect the different energy demands of bacilli growing logarithmically *in vitro* or inside the macrophage phagosome. However the differential regulation of genes encoding ATP synthase subunits may be involved in the maintenance of intracellular pH, or may represent the sudden requirement for metabolic activity as the bacilli adapt to a changing environment. This may explain the induction of *atpE/F/H* at 21 days after infection of immuno-competent compared to severe-immuno deficient (SCID) mice as the bacilli respond to the adaptive immune challenge in the immuno-competent environment [27].

Iron Sequestration

The lack of available iron during infection is a problem encountered by many pathogens, which is further exploited by the induction of iron-sequestering complexes as part of the host immune response [39]. *M. tuberculosis* genes involved in the generation of mycobactin, a siderophore-like iron-binding molecule (*mbtA-J*) were identified to be induced in IFN γ -activated murine macrophages [25]; a subset of genes up-regulated in low iron conditions were also induced in naïve murine macrophages. The induction of genes involved in the control of mycobacterial iron sequestration and storage has also been demonstrated to occur during murine infection; Timm *et al.* [30] reported the induction of *mbtB* in murine lungs, which coincided with the control of bacterial growth by the immune response. In addition the expression of *bfrA* (encoding an iron-storing bacterioferritin) was down-regulated [30]. This gene expression profile correlates well with the up-regulation of *mbtD* (involved in mycobactin synthesis), *hupB* (coding for an iron-regulated protein), and *fdxA* (encoding ferredoxin) at 21 days post infection in immuno-competent compared to severely immuno-deficient mice [27]. The *M. tuberculosis* genes *mbtB*, *mbtI* and *Rv3402c* regulated by IdeR (an iron-dependent regulator) were also induced on infection of human macrophage-like THP-1 cells [40]. Furthermore *furB*, encoding a probable ferric/zinc uptake regulator was also up-regulated on THP-1 infection [41]. The relative increase in the expression of genes involved in iron sequestration in activated macrophages (or immuno-competent mice) suggests that the bacilli find themselves in an iron-limited environment after immune activation, or that the mycobacterial requirement for iron increases in the microenvironment in which the bacilli reside.

In addition to iron, other moieties required for successful mycobacterial growth are likely to be limited at various stages of infection. Rachman *et al.* [19] observed an induction of amino acid transport-related genes in *M. tuberculosis* infected human lung sections. Indeed a number of genes identified to be induced by nutrient starvation were also demonstrated to be up-regulated after infection of naïve or activated murine macrophages [42, 25]. The

requirement of *relA* (encoding (p)ppGpp synthase and hydrolase activities) as part of the stringent response has been demonstrated in long-term anaerobic models [43] and during murine infection [31]. A comparison of the expression profiles of a *relA* knockout mutant with WT *M. tuberculosis* under starvation conditions has identified around 160 genes that may be regulated by (p)ppGpp, including genes involved in anaerobic respiration (*narH*, *narJ*) and fatty acid metabolism (*aceAb*, *acpM*, *fas*, *kasA*, *kasB*) [44]. Further exploration of the mycobacterial stringent response may reveal insights into how *M. tuberculosis* bacilli are able to adapt to nutrient-limited surroundings during infection.

Mycobacterial Lipid Moieties

The mycobacterial cell wall acts as the interface between the infecting bacilli and the host environment, therefore it is unsurprising to find genes involved in the synthesis and modification of cell wall associated fatty acids up-regulated *in vivo*. Genes involved in mycolic acid modification (*desA1/2* and *umaA*) were found to be induced inside the murine phagosome [25]. Additionally the desaturases encoded by *desA1* and *desA3* were up-regulated in infected human lung together with *mmaA3/A4* and *umaA* [19]. The induction of these genes is likely connected with the maintenance of the hydrophobic nature of the cell wall barrier from damage during macrophage infection. A second set of genes coding for polyketide synthases involved in the biosynthesis of mycobacterial complex lipids have also been demonstrated to be induced during macrophage infection. Mycocerosic acid synthase (*mas*) and the related genes *fadD28* [41] and *fadD26* [25] were up-regulated in the macrophage phagosome; these gene products are part of the biosynthetic pathway of phthiocerol dimycocerosate which is required for successful mycobacterial infection [45]. In addition the induction of *pks2* [32], necessary for sulpholipid generation [46], and *pks6* [33], involved in the expression of an unknown polar lipid [47], in human macrophages further highlights the potential importance of mycobacterial complex lipids during infection. Whether these lipid moieties are part of a response to strengthen the lipid-rich mycobacterial cell wall, or play a more active role in host-pathogen interactions is currently unknown [12].

DNA Repair

Mycobacterial genes involved in DNA recombination and repair were up-regulated in the intraphagosomal environment of both naïve and activated murine macrophages (*alkA*, *recX*, *recC*, *dinF* and *radA*). Interestingly this pattern of gene expression was also observed on infection of NOS2-deficient macrophages, indicating that the probable *M. tuberculosis* response to DNA damage occurs in the absence of nitric oxide [25]. A similar subset of genes *mfd*, *dinF/G* and *polA* were also induced on murine macrophage infection [28]. Furthermore genes involved in mycobacterial DNA repair have also been demonstrated to be up-regulated on infection of human macrophages (*uvrA*) [32], and the human lung (*dinX*, *dinF*, *gyrA/B*) [19].

Molecular Chaperones

The induction of molecular chaperones involved in the folding and translocation of polypeptides has been observed in *M. tuberculosis* under a number of stress conditions, exemplified by the heat shock response [48]. Rachman *et al.* [19] identified a number of genes related to chaperone biosynthetic pathways (*groEL/ES*, *suhB*, *dnaJ1/2*, *dnaK*, *hspR* and *hspX*) to be up-regulated in the human lung during *M. tuberculosis* infection. Similarly the induction of *groEL2* was reported after infection of human macrophage cell lines [41]. The activation of genes coding for molecular chaperones and DNA repair proteins may be part of a common stress response induced by multiple stimuli, for example different drug treatments [49, 50], the details of which are yet to be elucidated.

Antigen Expression

The induction of genes encoding members of the highly immunogenic PE/PE-PGRS and PPE families *in vivo* was demonstrated in human pulmonary tissue [19] and murine macrophages [25]. Interestingly the induction of some PE/PPE genes occurs alongside the repression of others in both macrophage and murine models [51], and this pattern of PE/PPE differential expression is repeated across many different growth/stress conditions [52]. The functional significance of these gene families, which command approximately 10% of the coding capacity of *M. tuberculosis* H37Rv is uncertain [53], however there is evidence that some of these proteins are found at the cell surface and may mediate interactions with the host [54]. Elucidation of the expression pattern of these genes, which may act as variable surface antigens during infection [55], may influence future vaccine design strategies. Other potential *M. tuberculosis* antigens such as members of the ESAT-6 family (*esxH*, *esxO*, *esxV*) were also identified to be expressed in the murine lung [56]. Shi *et al.* [57] identified differences in the transcription of genes encoding *M. tuberculosis* secreted antigens throughout murine lung infection, and Dahl *et al.* [44] have reported that the expression of many of these antigenic proteins may be under the control of *relA* as part of the mycobacterial stringent response. These observations highlight how gene expression approaches may be used to supplement antigenic studies and influence vaccine design strategy.

The Regulation of Mycobacterial *In Vivo* Gene Expression

A greater understanding of the regulation of *M. tuberculosis* gene expression may be gained by looking at the differential expression of genes known to control mycobacterial transcription. The induction of low iron responsive (*ideR*) and heat shock responsive (*hspR*) transcriptional regulators are likely at least in part to coordinate the expression of iron sequestration factors and molecular chaperones as discussed above. The transcription factors *sigB* and *sigE* were found to be induced in the murine phagosome [25]. One possible stimulus for the increased expression of these regulatory proteins has been demonstrated to be cell wall stress (treatment with detergent [58]), something that is likely to be relevant throughout infection. Furthermore *sigB*, *sigC* and *sigH* were observed to be up-regulated within artificial murine granulomas [31] and *sigE*, *sigG* and *sigH* have been demonstrated to be induced inside human macrophages [26, 32]. In addition, the regulatory proteins *sigF* and *sigL* may also be necessary for successful *in vivo* survival [33, 59, 60]. The up-regulation of a number of two component systems *in vivo* has also been reported, including *dosR* which mediates the *M. tuberculosis* response to hypoxia [23], and which is induced together with much of its regulon inside artificial murine granulomas [31] as previously described. Moreover the expression of *mtrA*, *regX3*, *phoP*, *prfA*, *mprA*, *kdpE*, *trcR* and *tcrX* have all been detected after infection of human-derived macrophages with *M. tuberculosis* [61, 62]. Further characterisation of the gene subsets controlled by these global regulators and of the stimuli that influence their expression will help reveal the networks of gene regulation required for *M. tuberculosis* pathogenicity.

What the *M. tuberculosis* Intracellular Transcriptome Can Tell us About the Phagosomal Environment

The comparison of expression data from *in vitro* and *in vivo* models is a valuable tool in understanding both the nature of the intracellular environment that *M. tuberculosis* encounters during infection and how bacilli respond to these stresses. By comparing overlapping gene expression patterns from *in vitro* and *in vivo* models it is possible to speculate on the conditions that infecting bacilli face. The phagosomal environment of naïve macrophages is likely to be low in glucose or glycerol based carbon sources, low in amino acids, and low in iron as compared to nutrient starvation and low iron *in vitro* conditions [42, 63, 64]. On activation (of murine macrophages with IFN γ) the bacilli are likely to be

exposed to nitric oxide, oxidative stress and possibly a hypoxic environment [25], although a shift to anaerobic respiration in bacilli may be due to the presence of NO, rather than the absence of oxygen [36]. Schnappinger *et al.* [25] defined 68 genes that were preferentially induced after infection of IFN γ -activated compared to naïve murine macrophages. Nearly half of these genes were identified as part of the *dosR* regulon, a set of around 50 genes induced by hypoxia [23, 35] and nitric oxide treatment [36]. Additionally many of the activation-specific genes were also up-regulated by nitric oxide or hydrogen peroxide treatment *in vitro*, and the induction of these genes was not observed in NOS2-deficient macrophages [25]. A subset of iron-responsive genes was also induced to a greater degree in activated murine macrophages. The intraphagosomal *M. tuberculosis* expression profile also encompasses subsets of genes that have been characterised as part of the mycobacterial responses to various stresses, such as DNA damage, detergent treatment, low pH and heat shock [48, 58, 65, 66]. A summary of the possible microenvironments that *M. tuberculosis* encounters during infection is depicted in Fig. (2).

Mycobacterial gene expression is regulated by many factors and controlled by a complex network of sensory systems and feedback loops. It is clear that some pathways are induced by multiple stimuli; for example genes associated with the mycobacterial response to low iron are also induced by nitric oxide, H₂O₂ or hypoxia [25, 35]. Furthermore the expression of *dosR* (integral to the *M. tuberculosis* response to macrophage activation and persistence as modelled *in vitro*) is induced by hypoxia, nitric oxide (s-nitrosoglutathione), ethanol and H₂O₂ treatments but not heat or cold-shock [67]. Further investigation is required to identify which environmental conditions are influencing *M. tuberculosis* gene expression in an effort to better understand the multi-factorial infection process.

INFECTION MODELS AND MAN

The comparison of *M. tuberculosis* gene expression profiles from tissue culture models derived from murine or human sources is challenging. This is partly due to differences in the survival of bacilli after macrophage infection between murine and human models. Schnappinger *et al.* [25] described little or no growth of *M. tuberculosis* in naïve murine bone marrow macrophages and a decrease in mycobacterial survival after 72 h in IFN γ -activated macrophages, compared to successful *M. tuberculosis* growth in human-derived monocytes and THP-1 cells [68, 69]. This may be due to variation in immunologically relevant elements, such as TLR expression [70], or the production of antimicrobial moieties such as nitric oxide. Indeed the role of nitric oxide in the control of human mycobacterial infection has been controversial; although it is likely that nitric oxide does play some part in the human immune response to *M. tuberculosis* [71]. The induction of nitric oxide from cells *in vitro* may however depend on where and how the immune cells were extracted, matured and infected [72].

The extrapolation of expression data from tissue culture models to animal models and natural *M. tuberculosis* infection is obviously complicated by many factors. Amongst the most apparent are the sampling of broad populations of bacilli from different microenvironments from *in vivo* infection models and the differences in pathology between murine and human infection [73]. There are however many similarities to be drawn between the mycobacterial responses to macrophage activation (derived from tissue culture models) and responses to the onset of the adaptive immune system in murine lungs; as expression profiling methods progress it should be possible to further explore human *M. tuberculosis* infection.

CONCLUSIONS

The transcriptional response of *M. tuberculosis* to the intracellular environment has highlighted a number of mechanisms that mycobacteria may utilise to successfully survive *in vivo*. This transcriptional data has also revealed a little about the microenvironments that bacilli may encounter. The future comparison of expression patterns from distinct mycobacterial populations from *in vivo*, tissue culture and *in vitro* models of infection promises to uncover a great deal more about *M. tuberculosis* pathogenesis, which will hopefully lead to novel treatment strategies.

One of the most exciting features of transcriptional profiling using DNA microarrays is the expression data obtained for genes of unknown function that would be overlooked using a bottom-up experimental strategy. As more microarray datasets become available the co-expression of genes encoding hypothetical proteins with known transcriptional pathways may allow novel gene functions to be described and explored. The transcriptional data discussed herein mostly concerns the induction of mycobacterial transcription. Nevertheless the repression of genes may be just as important for the outcome of host pathogen interactions during infection, as described recently for regulation of the *mce1* operon in murine macrophages [74]. The expression of mycobacterial genes discussed in this review is (in most cases) relative to *in vitro* logarithmically growing bacilli; this comparison highlights the differences between logarithmic growth in complete media and *in vivo* growth but does not necessarily reveal which mechanisms are key to mycobacterial survival (as discussed [75]), or indeed how essential particular genes are for successful infection. This review has focused purely on the mRNA expression data available for the intracellular survival of *M. tuberculosis*, ignoring the huge arsenal of data on the functional significance of particular genes using techniques such as signature tag mutagenesis [76, 77], and knockout/down studies [78, 79]; and of course areas including mRNA degradation, protein expression, processing and regulation.

We are currently able to study the transcriptome of a limited number of host-mycobacterial environments; the challenge for the next few years will be to integrate these datasets with expression data from additional infection models to elucidate host and tissue specific responses. The investigation of (relatively) simple *in vitro* or tissue culture models should help us to understand the transcriptional events from complex environments such as the murine or human lung. In addition the introduction of bacterial RNA amplification methods will allow the investigation of smaller, highly defined populations of bacilli from *in vivo*. Whole genome technologies offer exciting prospects for the future, such as the integration of expression data with transcriptional regulation data from emerging technologies such as ChIP/chip to identify global networks of expression that are important during infection; or in combination with gene knockout/knockdown technologies to investigate the effects of individual gene inactivation on the *M. tuberculosis* transcriptome during infection. All of this should aid in the understanding of *M. tuberculosis* natural infection and contribute to drug development programmes.

Acknowledgments

SJW was funded by an EU STREP Sixth Framework Programme Priority (LHP-CT-2004-012187). SJW and PDB would like to acknowledge the Wellcome Trust and its Functional Genomics Resources Initiative for funding the multi-collaborative microbial pathogen microarray facility at St. George's (BµG@S).

ABBREVIATIONS

RT-PCR Reverse transcription-polymerase chain reaction

TLR	Toll-like receptor
SCOTS	Selective capture of transcribed sequences
NOS2	Nitric oxide synthase 2

REFERENCES

- [1]. Chackerian AA, Alt JM, Perera TV, Dascher CC, Behar SM. *Infect. Immun.* 2002; 70:4501–4509. [PubMed: 12117962]
- [2]. Clemens DL, Horwitz MA. *J. Exp. Med.* 1995; 181:257–270. [PubMed: 7807006]
- [3]. Pieters J, Gatfield J. *Trends Microbiol.* 2002; 10:142–146. [PubMed: 11864824]
- [4]. Sturgill-Koszycki S, Schlesinger PH, Chakraborty P, Haddix PL, Collins HL, Fok AK, Allen RD, Gluck SL, Heuser J, Russell DG. *Science.* 1994; 263:678–681. [PubMed: 8303277]
- [5]. Deretic V, Fratti RA. *Mol. Microbiol.* 1999; 31:1603–1609. [PubMed: 10209735]
- [6]. Saunders BM, Frank AA, Orme IM. *Immunology.* 1999; 98:324–328. [PubMed: 10583589]
- [7]. Flynn JL, Chan J. *Annu. Rev. Immunol.* 2001; 19:93–129. [PubMed: 11244032]
- [8]. Saunders BM, Cooper AM. *Immunol. Cell Biol.* 2000; 78:334–341. [PubMed: 10947857]
- [9]. Russell DG. *Nat. Rev. Mol. Cell Biol.* 2001; 2:569–577. [PubMed: 11483990]
- [10]. Chan J, Flynn J. *Clin. Immunol.* 2004; 110:2–12. [PubMed: 14986673]
- [11]. Walburger A, Koul A, Ferrari G, Nguyen L, Prescianotto-Baschong C, Huygen K, Klebl B, Thompson C, Bacher G, Pieters J. *Science.* 2004; 304:1800–1804. [PubMed: 15155913]
- [12]. Beatty WL, Ullrich HJ, Russell DG. *Eur. J. Cell Biol.* 2001; 80:31–40. [PubMed: 11211933]
- [13]. Quesniaux V, Fremont C, Jacobs M, Parida S, Nicolle D, Yermeev V, Bihl F, Erard F, Botha T, Drennan M, Soler MN, Le Bert M, Schnyder B, Ryffel B. *Microbes Infect.* 2004; 6:946–959. [PubMed: 15310472]
- [14]. Hu Y, Mangan JA, Dhillon J, Sole KM, Mitchison DA, Butcher PD, Coates AR. *J. Bacteriol.* 2000; 182:6358–6365. [PubMed: 11053379]
- [15]. Pai SR, Actor JK, Sepulveda E, Hunter RL Jr, Jagannath C. *Microb. Pathog.* 2000; 28:335–342. [PubMed: 10839970]
- [16]. McKinney JD. *Nat. Med.* 2000; 6:1330–1333. [PubMed: 11100116]
- [17]. Gomez JE, McKinney JD. *Tuberculosis (Edinb).* 2004; 84:29–44. [PubMed: 14670344]
- [18]. Fenhalls G, Stevens L, Moses L, Bezuidenhout J, Betts JC, Helden P.v. Lukey PT, Duncan K. *Infect. Immun.* 2002; 70:6330–6338. [PubMed: 12379712]
- [19]. Rachman H, Strong M, Ulrichs T, Grode L, Schuchhardt J, Mollenkopf H, Kosmiadi GA, Eisenberg D, Kaufmann SH. *Infect. Immun.* 2006; 74:1233–1242. [PubMed: 16428773]
- [20]. Schnappinger D, Schoolnik GK, Ehrt S. *Microbes Infect.* 2006; 8:1132–1140. [PubMed: 16517202]
- [21]. McKinney JD, Gomez JE. *Nat. Med.* 2003; 9:1356–1357. [PubMed: 14595429]
- [22]. Kaufmann SH. *Nat. Rev. Immunol.* 2001; 1:20–30. [PubMed: 11905811]
- [23]. Park HD, Guinn KM, Harrell MI, Liao R, Voskuil MI, Tompa M, Schoolnik GK, Sherman DR. *Mol. Microbiol.* 2003; 48:833–843. [PubMed: 12694625]
- [24]. Muttucumar DG, Roberts G, Hinds J, Stabler RA, Parish T. *Tuberculosis (Edinb).* 2004; 84:239–246. [PubMed: 15207493]
- [25]. Schnappinger D, Ehrt S, Voskuil MI, Liu Y, Mangan JA, Monahan IM, Dolganov G, Efron B, Butcher PD, Nathan C, Schoolnik GK. *J. Exp. Med.* 2003; 198:693–704. [PubMed: 12953091]
- [26]. Cappelli G, Volpe E, Grassi M, Liseo B, Colizzi V, Mariani F. *Res. Microbiol.* 2006; 157:445–455. [PubMed: 16483748]
- [27]. Talaat AM, Lyons R, Howard ST, Johnston SA. *Proc. Natl. Acad. Sci. USA.* 2004; 101:4602–4607. [PubMed: 15070764]
- [28]. Rachman H, Strong M, Schaible U, Schuchhardt J, Hagens K, Mollenkopf H, Eisenberg D, Kaufmann SH. *Microbes Infect.* 2006; 8:747–757. [PubMed: 16513384]

- [29]. McKinney JD, Honer zu Bentrup K, Munoz-Elias EJ, Miczak A, Chen B, Chan WT, Swenson D, Sacchettini JC, Jacobs WR Jr, Russell DG. *Nature*. 2000; 406:735–738. [PubMed: 10963599]
- [30]. Timm J, Post FA, Bekker LG, Walther GB, Wainwright HC, Manganelli R, Chan WT, Tsenova L, Gold B, Smith I, Kaplan G, McKinney JD. *Proc. Natl. Acad. Sci. USA*. 2003; 100:14321–14326. [PubMed: 14623960]
- [31]. Karakousis PC, Yoshimatsu T, Lamichhane G, Woolwine SC, Nuermberger EL, Grosset J, Bishai WR. *J. Exp. Med.* 2004; 200:647–657. [PubMed: 15353557]
- [32]. Graham JE, Clark-Curtiss JE. *Proc. Natl. Acad. Sci. USA*. 1999; 96:11554–11559. [PubMed: 10500215]
- [33]. Li MS, Waddell SJ, Monahan IM, Mangan JA, Martin SL, Everett MJ, Butcher PD. *FEMS Microbiol. Lett.* 2004; 233:333–339. [PubMed: 15063504]
- [34]. Shi L, Sohaskey CD, Kana BD, Dawes S, North RJ, Mizrahi V, Gennaro ML. *Proc. Natl. Acad. Sci. USA*. 2005; 102:15629–15634. [PubMed: 16227431]
- [35]. Bacon J, James BW, Wernisch L, Williams A, Morley KA, Hatch GJ, Mangan JA, Hinds J, Stoker NG, Butcher PD, Marsh PD. *Tuberculosis (Edinb)*. 2004; 84:205–217. [PubMed: 15207490]
- [36]. Voskuil MI, Schnappinger D, Visconti KC, Harrell MI, Dolganov GM, Sherman DR, Schoolnik GK. *J. Exp. Med.* 2003; 198:705–713. [PubMed: 12953092]
- [37]. Shi L, Jung YJ, Tyagi S, Gennaro ML, North RJ. *Proc. Natl. Acad. Sci. USA*. 2003; 100:241–246. [PubMed: 12506197]
- [38]. Boshoff HI, Barry CE 3rd. *Nat. Rev. Microbiol.* 2005; 3:70–80. [PubMed: 15608701]
- [39]. Weinberg ED. *Life Sci.* 1992; 50:1289–1297. [PubMed: 1560730]
- [40]. Gold B, Rodriguez GM, Marras SA, Pentecost M, Smith I. *Mol. Microbiol.* 2001; 42:851–865. [PubMed: 11722747]
- [41]. Li MS, Monahan IM, Waddell SJ, Mangan JA, Martin SL, Everett MJ, Butcher PD. *Microbiology*. 2001; 147:2293–2305. [PubMed: 11496006]
- [42]. Betts JC, Lukey PT, Robb LC, McAdam RA, Duncan K. *Mol. Microbiol.* 2002; 43:717–731. [PubMed: 11929527]
- [43]. Primm TP, Andersen SJ, Mizrahi V, Avarbock D, Rubin H, Barry CE 3rd. *J. Bacteriol.* 2000; 182:4889–4898. [PubMed: 10940033]
- [44]. Dahl JL, Kraus CN, Boshoff HI, Doan B, Foley K, Avarbock D, Kaplan G, Mizrahi V, Rubin H, Barry CE 3rd. *Proc. Natl. Acad. Sci. USA*. 2003; 100:10026–10031. [PubMed: 12897239]
- [45]. Cox JS, Chen B, McNeil M, Jacobs WR Jr. *Nature*. 1999; 402:79–83. [PubMed: 10573420]
- [46]. Sirakova TD, Thirumala AK, Dubey VS, Sprecher H, Kolattukudy PE. *J. Biol. Chem.* 2001; 276:16833–16839. [PubMed: 11278910]
- [47]. Waddell SJ, Chung GA, Gibson KJ, Everett MJ, Minnikin DE, Besra GS, Butcher PD. *Lett. Appl. Microbiol.* 2005; 40:201–206. [PubMed: 15715645]
- [48]. Stewart GR, Wernisch L, Stabler R, Mangan JA, Hinds J, Laing KG, Young DB, Butcher PD. *Microbiology*. 2002; 148:3129–3138. [PubMed: 12368446]
- [49]. Boshoff HI, Myers TG, Copp BR, McNeil MR, Wilson MA, Barry CE 3rd. *J. Biol. Chem.* 2004; 279:40174–40184. [PubMed: 15247240]
- [50]. Waddell SJ, Stabler RA, Laing K, Kremer L, Reynolds RC, Besra GS. *Tuberculosis (Edinb)*. 2004; 84:263–274. [PubMed: 15207496]
- [51]. Dheenadhayalan V, Delogu G, Sanguinetti M, Fadda G, Brennan MJ. *J. Bacteriol.* 2006; 188:3721–3725. [PubMed: 16672626]
- [52]. Voskuil MI, Schnappinger D, Rutherford R, Liu Y, Schoolnik GK. *Tuberculosis (Edinb)*. 2004; 84:256–262. [PubMed: 15207495]
- [53]. Cole ST, Brosch R, Parkhill J, Garnier T, Churcher C, Harris D, Gordon SV, Eiglmeier K, Gas S, Barry CE 3rd, Tekaia F, Badcock K, Basham D, Brown D, Chillingworth T, Connor R, Davies R, Devlin K, Feltwell T, Gentles S, Hamlin N, Holroyd S, Hornsby T, Jagels K, Krogh A, McLean J, Moule S, Murphy L, Oliver K, Osborne J, Quail MA, Rajandream MA, Rogers J, Rutter S, Seeger K, Skelton J, Squares R, Squares S, Sulston JE, Taylor K, Whitehead S, Barrell BG. *Nature*. 1998; 393:537–544. [PubMed: 9634230]

- [54]. Brennan MJ, Delogu G, Chen Y, Bardarov S, Kriakov J, Alavi M, Jacobs WR Jr. *Infect. Immun.* 2001; 69:7326–7333. [PubMed: 11705904]
- [55]. Banu S, Honore N, Saint-Joanis B, Philpott D, Prevost MC, Cole ST. *Mol. Microbiol.* 2002; 44:9–19. [PubMed: 11967065]
- [56]. Dubnau E, Chan J, Mohan VP, Smith I. *Infect. Immun.* 2005; 73:3754–3757. [PubMed: 15908407]
- [57]. Shi L, North R, Gennaro ML. *Infect. Immun.* 2004; 72:2420–2424. [PubMed: 15039373]
- [58]. Manganelli R, Voskuil MI, Schoolnik GK, Smith I. *Mol. Microbiol.* 2001; 41:423–437. [PubMed: 11489128]
- [59]. Geiman DE, Kaushal D, Ko C, Tyagi S, Manabe YC, Schroeder BG, Fleischmann RD, Morrison NE, Converse PJ, Chen P, Bishai WR. *Infect. Immun.* 2004; 72:1733–1745. [PubMed: 14977982]
- [60]. Dainese E, Rodrigue S, Delogu G, Provvedi R, Laflamme L, Brzezinski R, Fadda G, Smith I, Gaudreau L, Palu G, Manganelli R. *Infect. Immun.* 2006; 74:2457–2461. [PubMed: 16552079]
- [61]. Zahrt TC, Deretic V. *Proc. Natl. Acad. Sci. USA.* 2001; 98:12706–12711. [PubMed: 11675502]
- [62]. Haydel SE, Clark-Curtiss JE. *FEMS Microbiol. Lett.* 2004; 236:341–347. [PubMed: 15251217]
- [63]. Rodriguez GM, Voskuil MI, Gold B, Schoolnik GK, Smith I. *Infect. Immun.* 2002; 70:3371–3381. [PubMed: 12065475]
- [64]. Hampshire T, Soneji S, Bacon J, James BW, Hinds J, Laing K, Stabler RA, Marsh PD, Butcher PD. *Tuberculosis (Edinb).* 2004; 84:228–238. [PubMed: 15207492]
- [65]. Rand L, Hinds J, Springer B, Sander P, Buxton RS, Davis EO. *Mol. Microbiol.* 2003; 50:1031–1042. [PubMed: 14617159]
- [66]. Fisher MA, Plikaytis BB, Shinnick TM. *J. Bacteriol.* 2002; 184:4025–4032. [PubMed: 12081975]
- [67]. Kendall SL, Movahedzadeh F, Rison SC, Wernisch L, Parish T, Duncan K, Betts JC, Stoker NG. *Tuberculosis (Edinb).* 2004; 84:247–255. [PubMed: 15207494]
- [68]. Stokes RW, Dooze D. *Cell Immunol.* 1999; 197:1–9. [PubMed: 10555990]
- [69]. Munoz-Elias EJ, McKinney JD. *Nat. Med.* 2005; 11:638–644. [PubMed: 15895072]
- [70]. Lauw FN, Caffrey DR, Golenbock DT. *Trends Immunol.* 2005; 26:509–511. [PubMed: 16111920]
- [71]. Nathan C, Shiloh MU. *Proc. Natl. Acad. Sci. USA.* 2000; 97:8841–8848. [PubMed: 10922044]
- [72]. Chan ED, Chan J, Schluger NW. *Am. J. Respir. Cell Mol. Biol.* 2001; 25:606–612. [PubMed: 11713103]
- [73]. Tsai MC, Chakravarty S, Zhu G, Xu J, Tanaka K, Koch C, Tufariello J, Flynn J, Chan J. *Cell Microbiol.* 2006; 8:218–232. [PubMed: 16441433]
- [74]. Casali N, White AM, Riley LW. *J. Bacteriol.* 2006; 188:441–449. [PubMed: 16385033]
- [75]. Kendall SL, Rison SC, Movahedzadeh F, Frita R, Stoker NG. *Trends Microbiol.* 2004; 12:537–544. [PubMed: 15539113]
- [76]. Camacho LR, Ensergueix D, Perez E, Gicquel B, Guilhot C. *Mol. Microbiol.* 1999; 34:257–267. [PubMed: 10564470]
- [77]. Sassetti CM, Boyd DH, Rubin EJ. *Proc. Natl. Acad. Sci. USA.* 2001; 98:12712–12717. [PubMed: 11606763]
- [78]. Parish T, Gordhan BG, McAdam RA, Duncan K, Mizrahi V, Stoker NG. *Microbiology.* 1999; 145:3497–3503. [PubMed: 10627047]
- [79]. Ehrt S, Guo XV, Hickey CM, Ryou M, Monteleone M, Riley LW, Schnappinger D. *Nucleic Acids Res.* 2005; 33:e21. [PubMed: 15687379]
- [80]. Dubnau E, Fontan P, Manganelli R, Soares-Appel S, Smith I. *Infect. Immun.* 2002; 70:2787–2795. [PubMed: 12010964]

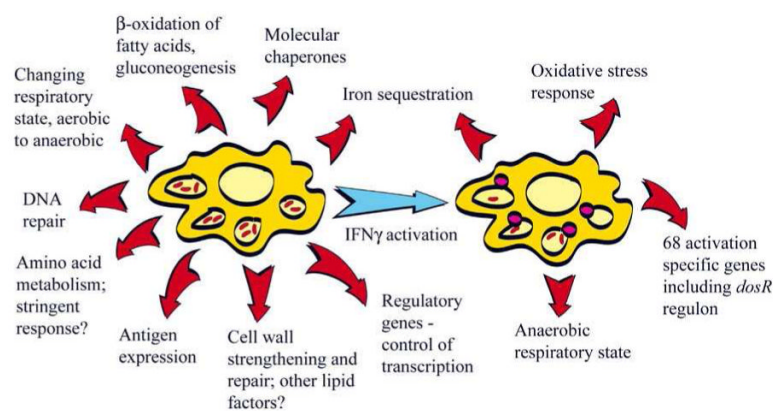


Fig. (1).

An illustration of the broad transcriptional responses of *M. tuberculosis* to the intracellular environments of naïve and IFN γ -activated macrophages. Macrophage activation (highlighted by phagolysosome fusion) exposes intracellular bacilli to nitric oxide, and results in decreased mycobacterial survival. Adapted from [21].

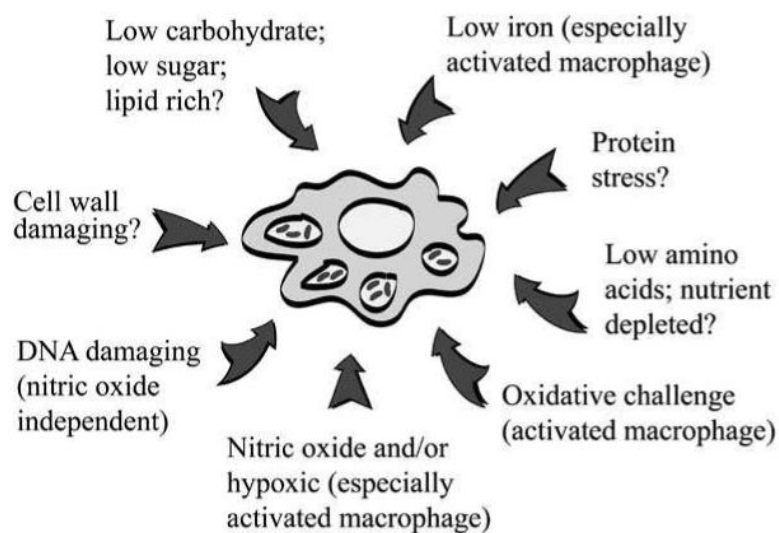


Fig. (2).

An illustration of the possible microenvironments that *M. tuberculosis* may encounter on infection as inferred from the transcriptional data discussed in this review.

Table 1

A List of Publications Describing the mRNA Expression of Multiple (5 or more) *M. tuberculosis* Genes from Intracellular Models of Infection, Detailing the Method Used and the Key Pathways Identified or Investigated. (mbRT-PCR – molecular beacon RT-PCR, SCOTS – selective capture of transcribed sequences)

Reference	Technique	<i>In vivo</i> model	Key pathways
Rachman <i>et al.</i> [19]	Membrane array	Human lung tissue	Cell wall/lipid synthesis Respiratory state
Fenhalls <i>et al.</i> [18]	<i>In situ</i> hybridisation	Human lung tissue	Fatty acid metabolism Respiratory state
Cappelli <i>et al.</i> [26]	Membrane array	Human peripheral blood derived monocytes	Transcriptional regulation Cell wall/lipid synthesis
Haydel and Clark-Curtiss [62]	SCOTS	Human peripheral blood derived monocytes	Transcriptional regulation
Graham and Clark-Curtiss [32]	SCOTS	Human peripheral blood derived monocytes	Fatty acid metabolism Transcriptional regulation
Li <i>et al.</i> [33]	Subtractive hybridisation to membrane array	THP-1 cells	Cell wall/lipid synthesis Transcriptional regulation
Dubnau <i>et al.</i> [80]	Promoter trap	THP-1 cells	Fatty acid metabolism Amino acid metabolism
Li <i>et al.</i> [41]	Subtractive hybridisation to membrane array	THP-1 cells	Cell wall/lipid synthesis Iron sequestration
Gold <i>et al.</i> [40]	mbRT-PCR	THP-1 cells	Iron sequestration
Dubnau <i>et al.</i> [56]	Promoter trap	C57BL/6 murine lung	Antigen expression Transcriptional regulation
Shi <i>et al.</i> [34]	mbRT-PCR	C57BL/6 murine lung	Respiratory state
Talaat <i>et al.</i> [27]	Microarray	BALB/c, SCID Murine lung	Fatty acid metabolism Iron sequestration
Shi <i>et al.</i> [37]	mbRT-PCR	C57BL/6 murine lung	Respiratory state
Voskuil <i>et al.</i> [36]	Quantitative RT-PCR	C57BL/6 murine lung	Respiratory state
Timm <i>et al.</i> [30]	Quantitative RT-PCR	C57BL/6 Murine lung, human lung tissue	Iron sequestration Fatty acid metabolism
Karakousis <i>et al.</i> [31]	Microarray	Artificial SKH1 murine granuloma	Respiratory state Amino acid metabolism
Rachman <i>et al.</i> [28]	Membrane array	Murine bone marrow derived macrophages	Amino acid metabolism Cell wall/lipid synthesis
Schnappinger <i>et al.</i> [25]	Microarray	Murine bone marrow derived macrophages	Fatty acid metabolism Respiratory state